201-15390

June 23, 2004

Michael O. Leavitt, Administrator
U.S. Environmental Protection Agency
Ariel Rios Bldg. (1101A)
1200 Pennsylvania Ave. NW
Washington, DC 20460

PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

HEADQUARTERS
501 FRONT ST.
NORFOLK, VA 23510
757-622-PETA
757-628-0781 (FAX)

Comments on the HPV test plan for diethyl ether

Dear Administrator Leavitt:

The following comments on the test plan for ethane 1,1'-oxybis, or diethyl ether (CAS no. 60-29-7), prepared by the Diethyl Ether Producers Association (DEEPA), are submitted on behalf of People for the Ethical Treatment of Animals, the Physicians Committee for Responsible Medicine, the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These animal, health, and environmental protection organizations have a combined membership of more than ten million Americans.

The DEEPA is proposing to conduct a combined repeated-dose, reproductive and developmental toxicity test (OECD no. 422) on diethyl ether. This test will kill at least 675 animals. We have the following criticisms of this plan.

First, we do not agree that the available data on the repeated-dose, developmental and reproductive toxicity of diethyl ether are unsatisfactory:

1. *Repeated-dose toxicity*

   The DEEPA lists a total of seven repeated-dose animal studies in its robust summaries, including 35-day inhalation studies in rats, mice and guinea pigs (IUCLID data set, pp. 42-45), 7-week inhalation studies in rats, guinea pigs and rabbits (IUCLID data set, pp. 45-46), and a 90-day gavage study in rats (IUCLID data set, p. 45). Three of these studies are judged to be "valid with restrictions" (IUCLID data set, pp. 43-45), yet the DEEPA states in its test plan, without explanation, that there is a "lack of available information," so yet another repeated-dose inhalation toxicity study will be carried out (test plan, p. 8). It judges the other four studies to be "not assignable" with respect to reliability (IUCLID data set, pp. 45-46), but does not provides an explanation for this judgment. It states that details about the 90-day rat study could not be found (test plan, p. 8), but more information could probably have been obtained simply by contacting American Biogenics Corp. (IUCLID data set, ref. 3)

2. *Developmental toxicity*

   The DEEPA lists four mammalian developmental toxicity studies (there was also one avian study; IUCLID data set, pp. 49-51), of which two were classified as "valid with restrictions" (for some reason, the DEEPA did not classify the other two studies). Yet,

again inexplicably, it then states that "no data were found that evaluated the reproductive or developmental toxicity of DEE" (test plan, p. 9). Developmental toxicity was demonstrated in all four studies, with diethyl ether being shown to cause fetal resorption, decreased fetal weight, decreased fetal bone length, and fetal hydronephrosis, hepatic abnormalities, generalized edema, and severe skeletal malformations (e.g. absence of sternum and vertebrae). Diethyl ether is therefore clearly a developmental toxicant, and should be regulated as such. The DEEPA's proposal appears to have as its goal the determination a no-effect-level for developmental toxicity, but this is emphatically not part of the HPV program. Furthermore, the DEEPA suggests that the severe developmental toxicity that was found was due to hypoxia (IUCLID data set, pp. 49-50, test plan, p. 9), yet the study proposed by the DEEPA involves administration by inhalation (test plan, p. 9), and the DEEPA provides no indication as to how the effects of hypoxia are to be excluded in this study if they could not be excluded in the previous studies.

3. *Reproductive toxicity*

The DEEPA refers to a male reproductive toxicity study (IUCLID data set, pp. 48-49), which was classed as "valid with restrictions." As with developmental toxicity, it is difficult to understand why they DEEPA then states that "No data were found that evaluated the reproductive or developmental toxicity of DEE" (test plan, p. 9). We have been unable to locate any information about female reproductive toxicity studies that have been carried out. However, as diethyl ether is a developmental toxicant, exposure of women of child-bearing potential to diethyl ether should be prevented or minimized, and its female reproductive toxicity is therefore purely academic.

To summarize, at least seven repeated-dose toxicity studies, four developmental toxicity studies, and one reproductive toxicity study have been carried out in rats, mice, guinea pigs, and rabbits, in addition to 15 acute toxicity and irritation studies in rats, mice, guinea pigs, rabbits, and dogs (IUCLID data set, pp. 34- 42). The responsibility for demonstrating that more essentially similar animal studies are required clearly lies with the sponsor. Yet the DEEPA fails to do so.

Secondly, the DEEPA does not appear to have made any attempt to use data from related compounds in order to predict the toxicity of diethyl ether. The use of structure-activity relationships in the analysis of toxicity has repeatedly been mandated by the EPA:

> Participants shall maximize the use of scientifically appropriate categories of related chemicals and structure activity relationships. (Wayland 1999)

The case for the use of structure-activity analysis is particularly strong in the case of aliphatic ethers, as the structure-activity relationships of these compounds have been thoroughly investigated, and the correlation between their toxic activities and molecular connectivity indices is known to be excellent (Di Paolo 1978a, 1978b). In particular, data from dimethyl ether could have been used to support the submission for diethyl ether, as the only difference between these two compounds is one additional methyl group in each alkyl chain length, which has perhaps the most readily predictable effect on toxicity of any difference in molecular structure. A test plan for dimethyl ether was submitted to the EPA by DuPont as part of the HPV challenge program

on November 20, 2000 (http://www.epa.gov/chemrtk/dimethr/c12794tc.htm), and no animal tests were proposed.

Thirdly, the number of Americans occupationally exposed to diethyl ether is more than 270,000 per year, of whom more than 35,000 are female (NIOSH). Diethyl ether is also still sometimes used as an anesthetic in some countries. Diethyl ether is therefore an almost ideal compound for the performance of exposure and epidemiology studies, but the DEEPA gives no consideration to this option, and provides little information about the use of or exposure to diethyl ether.

In addition to the repeated-dose, reproductive and developmental toxicity test, the DEEPA is planning to conduct an *in vitro* chromosomal aberration study (OECD no. 473) on diethyl ether. We urge the DEEPA to use either human lymphocytes or mammalian cells obtained from established cultures, so as to avoid killing additional animals in order to supply the cells for this test.

In summary, this test plan is a prime example of sloppy, thoughtless toxicology that ignores existing data and thus violates both the 1999 animal welfare agreement and the 2000 Federal register notice that state that "Participants shall maximize the use of existing and scientifically adequate data to minimize further testing" (Wayland 1999; *Federal Register* 2000). We urge the EPA and the DEEPA to withdraw the proposal to kill yet another 675 animals in the HPV program.

I can be reached at 757-622-7382, ext. 8001, or via e-mail at JessicaS@peta.org.

Sincerely,

Jessica Sandler
Federal Agency Liaison

References

Di Paolo, T., "Structure-activity relationships of anesthetic ethers using molecular connectivity", *Journal of Pharmacological Sciences*, 67: 564-566, 1978a.

Di Paolo, T., "Structure-activity relationships of anesthetic ethers using molecular connectivity", *Journal of Pharmacological Sciences*, 67: 566-568, 1978b.

Federal Register Vol. 65, No. 248, December 28, 2000.

NIOSH, *National Occupational Exposure Survey (1981-1983)*, http://www.cdc.gov/noes/noes1/32590sic.html.

Wayland, S.H., "Letters to manufacturers/importers", October 14, 1999, http://www.epa.gov/chemrtk/ceoltr2.htm.